Eshu (2011). ***Defining the molecular mechanism of hepatocyte growth factor- mediafeed immunoregulation dendritie cells.*** Ph.D. Thesis. CSIR-IMTECH, Chandigarh/ Jawaharlal Nehru University, New Delhi: India.
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SUMMARY OF THE THESIS

The maturation state of DCs is a key parameter that determines the balance between immunity versus tolerance. However, the maturation of DCs is largely influenced by the type of immunoregulators present in the extracellular milieu. Among various immunomodulators that regulate DC maturation and APC function, HGF is notable. Studies have shown that the level of HGF increases in various immunosuppressive conditions like tumors (Rutella et al., 2006a), and hematological malignancies including multiple MM and Hodgkin lymphoma (Seidel et al., 2002; Teofili et al., 2001). Furthermore, the increase in serum HGF levels weakens the antitumor efficacy of DC-based immunotherapy in MM patients by causing DC dysfunction (Rutella et al., 2006a). Indeed, HGF has been shown to induce DC tolerance (Benkhoucha et al., 2010). A clear understanding of the molecular mechanism involved in HGF-mediated immunoregulation of DCs is needed for developing future therapeutic strategies against these malignancies. Accordingly, the current study was initiated to define the molecular mechanism by which HGF regulates DCs.

The present study has primarily been done on BMDCs or sDCs of mouse origin. Results of some critical experiments were reconfirmed in huMoDCs. Our results establish that HGF inhibits NF-κB activation in DCs. This has been suggested by our observation demonstrating an inhibition of LPS induced-NF-κB DNA binding in DCs following HGF pretreatment. HGF inhibited DNA binding of all NF-κB complexes consisting of p65, p50, relB and cRel. The decrease in NF-κB DNA binding correlates with HGF-induced inhibition of IKK activity, and phosphorylation and degradation of IκB proteins.

HGF exhibits inhibitory effect on NF-κB signaling independent of the type of DC maturation stimulus. For example, stimulation with TNF-α, like LPS, also failed to activate NF-κB signaling in DCs. Furthermore, HGF-induced inhibition of NF-κB signaling was observed irrespective of cellular origin and genotype of DCs. In contrast to NF-κB signaling, activation of the MAPK pathway is not affected by HGF pretreatment in DCs, although HGF is known to regulate the MAPK pathway in some cell types (Rush et al., 2007, Awasthi and King, 2000). This discrepancy reflects the relative importance of the NF-κB pathway in HGF-regulated activation and effector functions of DCs versus other cell types. We further investigated the molecular mechanism by which HGF-induced c-MET signaling inhibition of the NF-κB pathway in DCs.

The sequence YVHVNATYVNV of c-MET has been shown to interact with PI3K in lung carcinoma cells (Ponzetto et al., 1993). Importantly, the PI3K/AKT pathway has been reported to inhibit NF-κB activation in DCs (Guha and Mackman, 2002; Sen et al., 2007). Therefore, the possibility that HGF activates the PI3K/AKT pathway was investigated. Our study demonstrates an induction of two distinct PI3K complexes p85α/p110α and p85α/p110δ following HGF stimulation of DCs. This causes activation of downstream effector, AKT.

The YVHVNATYVNV sequence also acts as a docking site for c-Src (Ponzetto et al., 1994). Additionally, c-Src is known to activate the PI3K/AKT pathway upon binding of double stranded RNA by TLR3 (Johnsen et al., 2006). Thus, the involvement of c-Src in HGF induced activation of PI3K/ AKT pathway was checked. We demonstrate here that HGF stimulation of DCs induces binding of c-Src to c-MET and concomitant activation of c-Src. Furthermore, activation of c-Src activation is required for HGF-induced recruitment of PI3K complexes to c-MET and induction of the PI3K/AKT pathway in DCs. Activation of c-Src-PI3K-AKT signaling cascade by HGF in turn leads to activation of downstream effector mTOR. However, HGF mediates mTOR activation by inducing phosphorylation/inactivation of GSK3β. The latter functions downstream of AKT, and upstream of mTOR in HGF-induced signaling pathway in DCs.

Because HGF inhibits activation of the NF-κB activation pathway in DCs, a role for the c-Src-PI3K-AKT-mTOR pathway in HGF-induced inhibition of NF-κB activation in DCs was determined. Experiments by using the pharmacological inhibitors of c-Src, PI3K and mTOR; and siRNAs specific for these signaling molecules showed that HGF exhibits inhibitory effect on NF-κB pathway in DCs via the c-Src-PI3K-mTOR pathway. In addition, PI3K complexes, p85α/p110α and p85α/p110δ play essential role in HGF-induced activation of mTOR, which is required for inhibition of NF-κB signaling in DCs. Although GSK3β also participates in HGF-induced signaling events in DCs, our results suggest that GSK3β probably plays an indirect role in HGF-induced inhibition of NF-κB signaling by promoting mTOR activation.

We further determined whether HGF regulates DC activation via the c-Src-PI3K-AKT-mTOR pathway. Accordingly, the effect of HGF pretreatment of BMDCs on LPS-stimulated expression of costimulatory molecules and secretion of proinflammatory

cytokines, IL-12p70 and TNF-α was investigated. LPS-induced upregulation of costimulatory molecules and secretion of proinflammatory cytokines was found to be inhibited by HGF pretreatment. However, the inhibitory effects of HGF on up-regulation of costimulatory molecules and secretion of proinflammatory cytokines were markedly reduced in BMDCs pretreated with c-Src, PI3K or mTOR inhibitor. Furthermore, BMDCs transfected with c-Src- or mTOR-specific siRNA continued to secrete IL-12p70 upon LPS stimulation despite HGF pretreatment. Collectively, these results suggest that HGF induces DC inhibition via activation of the c-Src-PI3K-AKT-mTOR pathway.

Our efforts were further directed to identify novel proximal effector(s) of HGF/c-MET signaling that mediates the inhibitory effect of HGF on DCs. HGF is known to recruit many SH2 domain-containing signaling molecules to c-MET. Notably, Btk, a member of Tec nonreceptor protein tyrosine kinase family, also contains SH2 domain and is involved in inhibition of LPS-induced DC maturation (Kawakami et al., 2006; Tzeng et al., 2000). However, the role of Btk in HGF-induced signaling is currently unknown. Accordingly, a role for Btk in HGF-induced inhibition of DCs was investigated. Our current study demonstrates that HGF induces Btk activation in DCs. Furthermore, HGF stimulation induces a physical association between Btk and c-MET receptor. Additionally Btk has been demonstrated here as an upstream regulator of c-Src activation in HGF-induced signaling in DCs. In fact, blockade of Btk activation prevents activation of c-Src and its downstream effectors such as PI3K and mTOR. In addition, Btk is required for HGF induced recruitment of c-Src, and PI3K complexes p85α/p110α and p85α/p110δ to c-MET.

Because Btk is an upstream regulator of the c-Src-PI3K-AKT-mTOR pathway, a role for Btk in HGF-induced inhibition of NF-κB signaling was determined in both murine DCs and huMoDCs. Our resuts show that Btk is required for HGF inhibition of LPS-induced NF-κB DNA binding, degradation of IκB proteins and IKK activity in DCs irrespective of cellular origin and genotype of DCs.

Notably, the role of Btk in HGF-induced inhibition of NF-κB signaling also correlated with its role in DC suppression caused by HGF. For example, inhibitor and siRNA-specific for Btk prevented HGF-induced inhibition of DCs. This suggested that DC inhibition by HGF is dependent on Btk activation. Furthermore, the current study demonstrates for the first time that HGF inhibits DC activation by inducing autocrine IL-

10 secretion, which requires activation of Btk and its downstream the c-Src-PI3K-AKT-mTOR pathway. Blocking activation of the Btk-c-Src-PI3K-AKT-mTOR pathway prevents HGF-induced IL-10 secretion by DCs. Furthermore, neutralization of IL-10 secretion from DCs blocks the inhibitory effect of HGF on DCs. Together, the current study unravels the molecular mechanism involved in HGF-mediated immunoregulation of DCs and establishes that HGF inhibits DC activation by inducing the Btk-c-Src-PI3K-AKT-mTOR pathway that blocks NF-κB signaling.

Our findings are summarized in Fig. 4.23

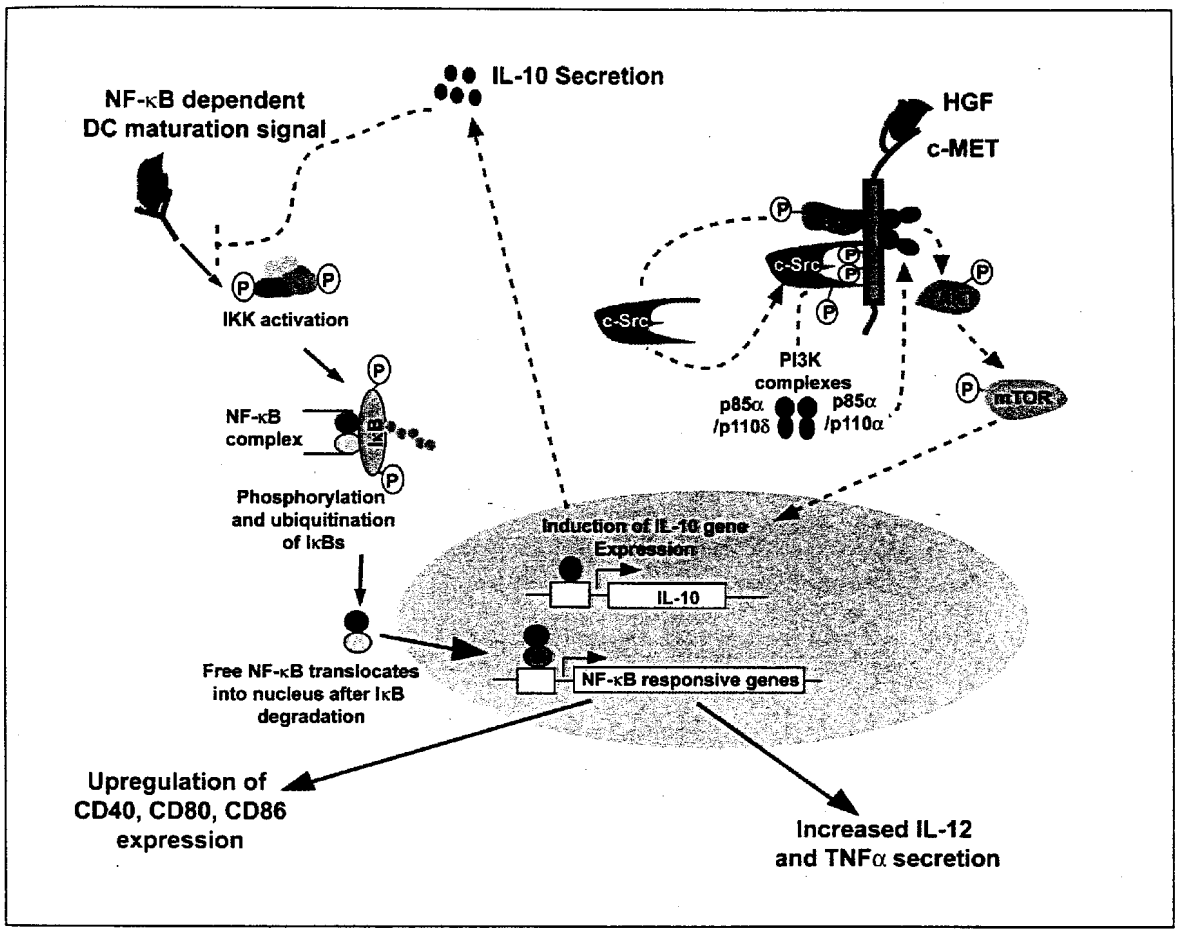

Fig. 4.23 Model depicting the molecular mechanism of HGF-mediated immunoregulation of DCs